IN THE TITLE:

Please amend the title to read:

SPECIFIC BINDING MOLECULES FOR THE ED-B DOMAIN OF FIBRONECTIN.

IN THE SPECIFICATION:

Please delete the first sentence after the title which was added in the preliminary amendment of April 28, 1999 and insert:

This application is a continuation-in-part of U.S. Application Serial No. 09/075,338 filed on May 11, 1998.

Please amend the following starting on page 10, line 20 and ending on page 10, line 31:

~~Detailed Description of the Drawings~~Details

Figure 1 shows:

Designed antibody phage library. ~~(a)~~Figure 1A Antibody fragments are displayed on phage as pIII fusion, as schematically depicted. In the antibody binding site (antigen's eye view), the Vk CDRs backbone is in yellow, the VH CDR backbone is in blue. Residues subject to random mutation are Vk CDR3 positions 91, 93, 94 and 96 (yellow), and VH CDR3 positions 95, 96, 97, and 98 (blue). The Cb atoms of these side chains are shown in darker colours. Also shown (in grey), are the residues of CDR1 and CDR2, which can be mutated to improve antibody affinity. Using the program RasMol (Chemistry Department website at U.C.S.C.) ~~(http://www.chemistry.ucsc.edu/wipke/teaching/rasmol.html)~~ the structure of the scFv were modeled from pdb file 1igm (Brookhaven Protein Data Bank; European Bioinformatics Institute website) ~~(http://www2.ebi.ac.uk/pcserv/pdbdb.htm)~~. ~~(b)~~Figure 1B PCR amplification and library

Please amend the following starting on page 11, line 7 and ending on line 13:

2D gels and western blotting ~~(a)~~Figure 2A Silver-staining of the 2D-PAGE of a lysate of human melanoma COLO-38 cells, to which recombinant ED-B-containing 7B89 had been added. The two 7B89 spots (circle) are due to partial proteolysis of the His-tag used for protein purification. ~~(b)~~Figure 2B Immunoblot of a gel, identical to the one of Fig. ~~2a~~2A, using the anti-ED-B E1 (Table 1) and the M2 anti-FLAG antibodies as detecting reagent. Only the 7B89 spots are detected, confirming the specificity of the recombinant antibody isolated from a gel spot.

Please amend the following starting on page 11, line 15 and ending on line 17:

Immunohistochemical experiments on serial sections of glioblastoma multiforme showing the typical glomerulus-like vascular structures stained using scFvs E1 ~~(A), A2(B)~~ (Figure 3A), A2 (Figure 3B), and G4 ~~(C)~~(Figure 3C). Scale bars: 20 μm.

Please amend the following starting on page 11, line 19 and ending on line 25:

Stability of antibody-(ED-B) complexes. Analysis of the binding of scFvs E1, H10 and L19 to the ED-B domain of fibronectin. ~~(a)~~Figure 4A BIAcore sensograms, showing the improved dissociation profiles obtained upon antibody affinity-maturation. ~~(b)~~Figure 4B Native gel electrophoretic analysis of scFv-(ED-B) complexes. Only the high-affinity antibody L19 can form a stable complex with the fluorescently labeled antigen. Fluorescence detection was performed as described (Neri et al. (1996) BioTechniques, 20, 708-712).

Please amend the following starting on page 11, line 26 and ending on line 29:

~~(c)~~Figure 4C Competition of the scFv-(ED-B-biotin) complex with a 100-fold molar excess of unbiotinylated ED-B, monitored by electrochemiluminescence using an Origen apparatus. A long half-life for the L19-(ED-B) complex can be observed. Black squares: L19; Open triangles: H10.

Please amend the fourth full paragraph on page 12 as follows:

Figure 9) shows immunohistochemical studies of ocular structures using the L19 antibody. A specific red staining is observed around neovascular structures in the cornea ~~(a)~~Figure 9A, but not around blood vessels in the iris ~~(b)~~Figure 9B and in the conjunctiva ~~(c)~~Figure 9C. Small arrows: corneal epithelium. Relevant blood vessels are indicated with large arrows. Scale bars: 50 μm

Please amend the fifth full paragraph on page 12 as follows:

Figure 10) shows immunophotodetection of fluorescently labeled antibodies targeting ocular angiogenesis. A strongly fluorescent corneal neovacsularisation (indicated by an arrow) is observed in rabbits injected with the antibody conjugate L19-Cy5~~(a)~~ (Figure 10A), specific for the ED-B domain of FN, but not with the antibody HyHEL-10-Cy5 ~~(b)~~ (Figure 10B). Immunofluorescence microscopy on cornea sections confirmed that L19-Cy5 ~~(c)~~ (Figure 10C), but not HyHEL-10-Cy5 ~~(d)~~ (Figure 10D) localizes around neovascular structures in the cornea. Images ~~(a,b)~~ (Figure 10A, B) were acquired 8 h after antibody injection; ~~(c, d)~~ (Figure 10C, D) were obtained using cornea sections isolated from rabbits 24 h after antibody injection. P, pellet.

Please amend the sixth paragraph starting on page 12, line 24 and ending on page 13, line 11 as follows:

Figure 11) shows macroscopic images of eyes of rabbits treated with photosensitiser conjugates. Eye of rabbit injected with L19-PS before ~~(a)~~Figure 11A and 16 h after irradiation with red light ~~(b)~~Figure 11B. The arrow indicates coagulated neovasculature, which is confirmed as a hypoflurescent area in the Cy5 fluoroangiogram of panel ~~(c)~~Figure 11C 16 h after irradiation. Note that no coagulation is observed in other vascular structures, for example in the dilated conjuctival vessels. For comparison, a Cy5 fluoroangiogram with hyperfluorescence of leaky vessels, and the corresponding colour photograph of untreated rabbit eye are shown in ~~(d)~~ Figure 11D and ~~(h)~~ Figure 11H. Pictures ~~(e,f,g)~~ (Figures 11E, 11F, 11G) are analogous to ~~(a,b,c)~~ Figures 11A, 11B, 11C but correspond to a rabbit injected with ovalbumin-PS and irradiated with red light. No coagulation can be observed, and the angiogram reveals hyperfluorescence of leaky vessels. The eyes of rabbits with early-stage angiogenesis and injected with L19-PS are shown in ~~(i-l)~~ Figures 11I-11L). Images before ~~(i)~~ Figure 11I and 16 h after irradiation with red light ~~(j)~~ Figure 11J reveal extensive and selective light-induced intravascular coagulation (arrow). Vessel occlusion (arrow) is particularly evident in the irradiated eye ~~(l)~~ Figure 11L of a rabbit immediately after euthanasia, but cannot be detected in the non-irradiated eye ~~(k)~~ Figure 11K of the same rabbit. P, pellet. Arrowheads indicate the corneo-scleral junction (limbus). In all

figures, dilated pre-existing conjunctival vessels are visible above the limbus, whereas growth of corneal neovascularisation can be observed from the limbus towards the pellet (P).

Please amend the first full paragraph starting on page 13, line 12 as follows:

Figure 12) shows microscopic analysis of selective blood vessel occlusion. H/E sections of corneas (a,e,b,f: non-fixed; i,j: paraformaldehyde fixed) of rabbits injected with ovalbumin-PS ~~(a,e,i)~~ (Figures 12A, 12E, 12I) or L19-PS ~~(b,f,j)~~ (Figures 12B, 12F, 12J) and irradiated. Large arrows indicated representative non damaged ~~(e,i)~~ (Figures 12E, 12I) or completely occluded ~~(f,j)~~ (Figures 12F, 12J) blood vessels. In contrast to the selective occlusion of corneal neovasculature and restricted perivascular damage (eosinophlia) mediated by L19-PS after irradiation ~~(b,f,j)~~ (Figures 12B, 12F, 12J) vessels in the conjunctiva ~~(k)~~ (Figure 12K) and iris ~~(l)~~ (Figure 12L) do not show sign of damage in the same rabbit. Fluorescent TUNEL assay indicates the different number of apoptotic cells in sections of irradiated rabbits injected with L19-PS ~~(c,g)~~ (Figures 12C, 12G) or with ovalbumin-PS ~~(d,h)~~ (Figures 12D, 12H). Large arrows indicate some relevant vascular structures. Small arrows indicate corneal epithelium. Scale bars: 100μm ~~(a,d)~~ (Figures 12A, 12D) and 25μm ~~(e-l)~~ (Figures 12E-12L)

Please cancel Page 27 and replace with new Page 27, attached.

Table 1:

Sequences of selected anti-ED-B antibody clones

	VH chain			VL chain		
Clone	31-33*	50-54*	95-98*	32*	50*	91-96*
A2	SYA	AISGSG	GLSI	Y	G	NGWYPW
G4	SYA	AISGSG	SFSF	Y	G	GGWLPY
E1	SYA	AISGSG	FPFY	Y	G	TGRIPP
H10	SFS	SIRGSS	FPFY	Y	G	TGRIPP
L19	SFS	SIRGSS	FPFY	Y	Y	TGRIPP

Relevant amino acid positions (*: numbering according to Tomlinson et al. (1995) EMBO J., 14, 4628-4638) of antibody clones isolated from the designed synthetic libraries. Single amino acid codes are used according to standard IUPAC nomenclature.